Bacteriological profile of ready-to-eat food items in Kathmandu

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Abstract: Ready-to-eat (RTE) food items are defined as the foods prepared or cooked in advance, with no further cooking or preparation required before being eaten. Consumption of ready-to-eat foods prepared in an unhygienic environment can lead to different types of food-borne illnesses ranging from mild self-limiting ones to severe food poisoning leading to mortality. The goal of this study was to see if the ready-to-eat foods available in Kathmandu was contaminated with bacteria and to determine the antibiotic susceptibility pattern of the isolated bacteria. A total of 100 ready-to-eat foods (momo & sauce, chowmein, pizza, thakali set, burger and samosa) were gathered from various locations around Kathmandu and analyzed by standard microbiological methods in the Microbiology Department of Tri-Chandra Multiple Campus from March to August 2022. Out of 100 samples analyzed, 55 showed the presence of bacteria; *Escherichia coli* was found to be predominant (52.72%), followed by *Bacillus cereus* (16.38%) and *Salmonella* spp (12.72%), among others. Momo and its sauce contained highest number of bacteria, followed by chowmein and burger; the least number of bacteria was found in samosa and pizza. Antibiotic susceptibility test was performed against all isolated bacteria following standard disc diffusion method. The bacterial isolates were sensitive towards Meropenem and Ceftriazone whereas they were resistant towards Ampicillin and Nalidixic acid. One bacterial isolate (*Klebsiella pneumoniae*) showed Multi Drug Resistance (MDR) which was also an ESBL (Extended Spectrum Beta Lactamase) producer. Significant presence of bacteria in ready-to-eat food items indicate the unhygienic practice of food preparation and handling and can pose threat to the health of general public. It also emphasizes the need for regular monitoring of food items to reduce the risk of food associated illnesses.

Keywords: Antibiotic susceptibility; Bacteria; Food-borne; Ready-to-eat.

Introduction

Foodborne diseases caused by different microorganisms like bacteria, viruses and protozoans are important because they lead to considerable instances of morbidity and mortality. In all nations of the world, including Nepal, regular occurrences of foodborne diseases are seen in the form of outbreaks or sporadic cases.[1] The foodborne diseases caused by enteric pathogens are mainly of three types: diarrheal diseases caused by *Campylobacter* spp., *Cryptosporidium* spp., *Entamoeba histolytica*, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), *Giardia lamblia*, norovirus, nontyphoidal Salmonella enterica, Shigella spp, Vibrio colerae, Shiga toxin producing E. coli; intoxications caused by Bacillus cereus, Clostridium perfringens, Staphylococcus aureus and Clostridium botulinum and invasive enteric disease caused by Hepatitis A virus, typhoidal and nontyphoidal Salmonella enterica, invasive nontyphoidal Salmonella enterica, Mycobacterium bovis, Brucella spp and Listeria spp.[1]

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The first ever done estimates of the global burden of food-borne infections present that almost 1 in 10 people get sick and 420,000 die each year after consuming contaminated food. Undercooked or raw food products like eggs, fresh foods and dairy products which are contaminated may cause diarrheal diseases which account for more than half of the global food-borne diseases' burden.[2] Many parts of the developing countries in the world with improper sanitation practices and lack of temperature control of food have higher frequencies of food-borne diseases.[3] Since the kitchen is where food is prepared and stored, infections are mostly harbored and spread here. Germs may be found on just about anything in the kitchen, including the sink, sponges, cutting boards, cooking utensils, refrigerator, and towels.[4]

Food items may be contaminated if there is any mistreatment during food preparation and processing that creates a favorable habitat for bacteria. When undesired contaminating bacteria multiplies, it degrades the sensory and organoleptic qualities of food and also contributes to sickness. Most of the pathogenic bacteria identified in food items are intestinal in origin, however certain pathogens can also be found on the skin, in the throat, and in nasal passages. Therefore, food handlers are accountable for contamination and cross-contamination. Food-borne illnesses mostly cause acute, moderate, and self-limiting gastroenteritis. Due to the ingestion of food that has been contaminated by microorganisms, food-borne illnesses manifest symptoms including nausea, vomiting, and diarrhea. Foodborne illnesses, which affect the immunological, respiratory, cardiovascular, and musculoskeletal systems, can also cause instances of chronic consequences.[2, 5, 6, 7]

The estimates on the number of foodborne disease occurrences, sequelae, deaths caused by food related illnesses, or the Disability Adjusted Life Years (DALYs) resulting from foodborne diseases at the national level[1] are not available in many countries like Nepal.[8,9]

Food items that can be consumed immediately at the point of sale without any need to be cooked, heated or chilled but can be further subjected to heat treatment or re-heating is known as ready-to-eat (RTE) food items. Ready-to-eat foods like street foods have become very appealing to the consumers because they are available at low cost, convenient, tastier and familiar.[10]

The aim of this study is to assess the bacteriological quality of popular ready-to-eat foods available in Kathmandu and to determine the antibiotic susceptibility pattern of the bacterial isolates.

Materials and Methods

Research Method

The research method was quantitative, and primary data were collected from March 2022 to August 2022 from different local hotels inside Kathmandu. Samples were processed in the Microbiology laboratory of Tri-Chandra Multiple Campus.

Study Variables

The variables of the study were different ready-to-eat food items, occurrence of bacteria, antibiotic susceptibility profile and Multi-drug resistant (MDR) bacteria.

Research Design

The study was field-based and cross-sectional.

Study/Sampling Site and Its Justification

Convenience sampling technique was used to collect the ready-to-eat food samples from March 2022 to May 2022. The samples were collected randomly from different local hotels in Sinamangal, New Baneshwar, Thamel, Maharajgunj, Budhanilkantha, Maitidevi, Old Baneshwar, Thimi, Gwarko, Ratnapark and Balaju areas of Kathmandu valley. The food items were chosen on the basis of the most commonly purchased food items by the local people.

Very little information is available about the microbiology of ready-to-eat food items available in Kathmandu or the

antibiotic susceptibility pattern of the isolates; hence, this study site has been selected for research.

Sample Size

A total of 100 samples (ready-to-eat food items) were collected from different hotels located in Kathmandu.

Sample Collection and Transport

Commonly consumed ready-to-eat food items by the people in Kathmandu such as momo (dumpling) and its sauce, chowmein (fried noodles), burger, samosa (fried vegetable and potato mix covered with flour wrap), pizza and Nepali local food set (commonly referred to as khana/thakali set containing rice, lentils, vegetables) were collected in a convenience sampling method. Food samples (100g each) were collected aseptically during lunch time in a sterile glass container with the help of a sterile spoon. The samples were then screw-capped, clearly labeled, and transported to the laboratory maintaining a cold chain, and immediately, to avoid contamination.

Sample Processing

Isolation, enumeration and identification of bacteria

Solid food items were weighed (25g each) and dispensed into conical flask containing 225 ml sterile Phosphate Buffered Saline (PBS) so that the dilution becomes 10^{-1}. The sample was mixed thoroughly by shaking for 5-10 minutes in aseptic condition. Serial dilution of the sample was then performed up to 10^{-6} dilutions. Similar procedure was employed for the liquid samples, i.e. the sauce of the momos. The bacterial load of the collected food samples was determined from different dilutions to come up with the enumeration of bacteria in the samples. Plate Count Agar (PCA) medium was used for the isolation and enumeration. Selective media like Eosin Methylene Blue (EMB) agar and Mannitol Salt Agar (MSA) was used for the isolation of *Escherichia coli* and *Staphylococcus aureus* respectively. MacConkey Agar (MA) was used for Gram negative bacteria.[11]

Bacterial colonies having greenish metallic sheen and dark center in EMB agar are indicative of *E. coli*. Mannitol fermenting (yellow) colonies from MSA subcultured on nutrient agar and incubated at 37°C for 24 hours gave rise to golden yellow colonies agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2–3 mm and were indicative of *S. aureus* (Fig. 1a). Further phenotypic identification of the bacteria was made by Gram staining, and appropriate biochemical tests like catalase, oxidase, oxidative/fermentative (O/F), Indole, Methyl Red, Voges Proskauer, and Citrate (IMViC tests), Triple Sugar Iron Agar (TSIA) test, Urease and coagulase test (slide and tube test) (Fig. 1b).

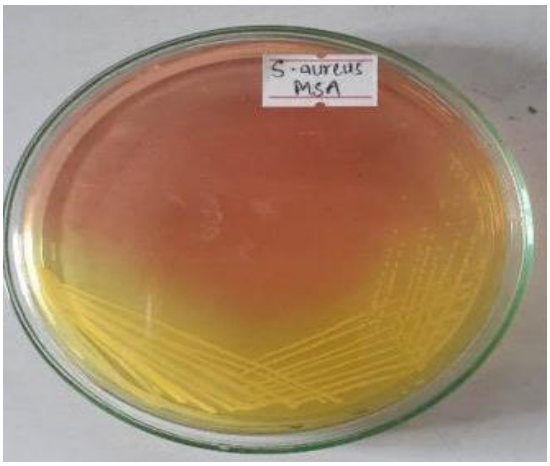

Fig 1a: *Staphylococcus aureus* colonies in Mannitol Salt Agar Agar.

Fig 1b: Biochemical test results of *Pseudomonas aeruginosa* (IMViC: --++; TSI: Alk/Alk, gas-ve, H_2S-ve; Urease:-ve; O/F: oxidative).

Antibiotic susceptibility testing by disc diffusion method

All the seven genera of identified bacterial isolates were subjected to in vitro antibiotic susceptibility tests by the modified Kirby Bauer disc diffusion method recommended by CLSI guidelines.[11] Some of the antibiotics tested were ampicillin (10 μg), amoxicillin- clavulanic acid (30 μg), ampicillin-sulbactam (10/10 μg), ciprofloxacin (5 μg), azithromycin (15 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), meropenem (10 μg), nitrofurantoin (300 μg). Briefly, the inoculums were prepared by transferring 3–4 identical colonies from the nutrient agar to sterile normal saline. The turbidity of the inoculums was made equivalent to a 0.5 McFarland standard. The lawn culture of the test inoculums was prepared by swabbing Mueller-Hinton agar (MHA) with a sterile cotton swab dipped into inoculums. Antibiotic discs were applied to the inoculated MHA plate and incubated at 37°C for 18 hours. After incubation, the zone of inhibition around the discs was noted, and the results were interpreted as sensitive, intermediate, or resistant.[12] (Fig. 2).

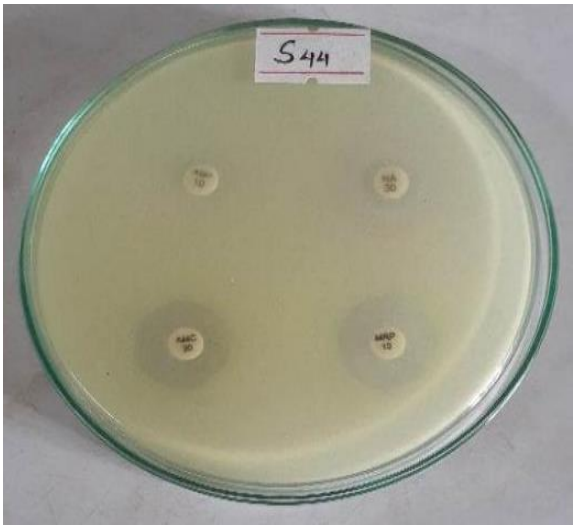

Fig. 2: Antibacterial susceptibility test of *Salmonella* spp: resistant to Ampicillin & Nalidixic Acid (upper half) and sensitive to Amoxicillin-clavulanic acid and Meropenem (lower half).

Detection of Multiple Drug Resistance Pattern

Antimicrobial resistance exhibited by a species of bacteria to at least one antimicrobial drug falling in three or more antimicrobial categories is considered as multiple drug resistance. Testing for multiple drug resistance was done for the 5 Gram negative bacterial species detected in this study.

Detection of Extended Spectrum *β*-lactamase (ESBL) producers

Extended Spectrum β-Lactamase production test was performed for multi-drug resistant (MDR) Gram negative bacteria. Antibiotics Ceftazidime (CAZ), Ceftazidime/Clavulanic acid (CAC) 30/10mg, cefotaxime (CTX) and Cefotaxime/Clavulanic acid (CEC) 30/10mg were used for this test. Phenotypic confirmation of ESBL was performed by disc diffusion test with confluent growth on Mueller Hinton Agar (MHA); a difference of 5 mm or more between the zone diameters of either of the cephalosporin discs and their corresponding cephalosporin/clavulanate discs confirms ESBL production. A lawn culture was done from the culture in nutrient broth and matched with 0.5 Mc Farland Solution; the 4 antibiotics were placed aseptically and incubated at 37°C for 24 hours (Fig. 3).

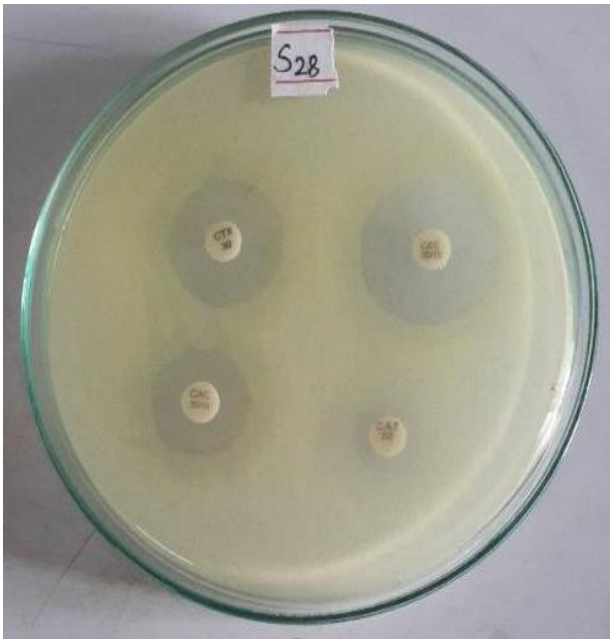

Fig. 3: Production of ESBL by Klebsiella pneumoniae: Cefotaxime (CTX) & Cefotaxime/clavulanic acid (CEC) in upper half and Ceftazidime/clavulanic acid (CAC) & Ceftazidime/(CAZ) in lower half.

Results and Discussion

Out of 100 ready-to-eat food samples processed, 55 samples showed growth of bacteria indicating contamination of food items. The samples collected from Ratnapark showed highest contamination whereas the lowest incidence of bacterial contamination was found in samples collected from New Baneshwar. Ratnapark is the central part of Kathmandu with major transportation junction and many people coming from different parts of the valley for commute and other purposes. High incidence of contamination of foods items can pose threat to people consuming ready-to-eat items in this area.

Among the different bacterial isolates obtained from the positive samples, 72.22% (n=40) were Gram negative and 27.78% (n=15) were Gram positive bacteria.

Table 1 shows that *Escherichia coli* was the prominent bacteria isolated from different food samples analyzed (53%) followed by *Bacillus cereus* (16%) and *Salmonella* spp. (13%). Only one isolate *Klebsiella pneumoniae* and *Enterobacter* spp (2% each) were identified in the tested food samples.

Table 1: Distribution of isolated bacteria in different food samples analyzed.

Food Samples → Bacteria isolated (n)↓	Momo (3)	Momo's sauce (14)	Chowmein (11)	Pizza (5)	Thakali set (6)	Burger (11)	Samosa (5)
Escherichia coli	3	9	6	1	4	3	3
Salmonella spp	0	3	2	0	0	1	1
Klebsiella pneumoniae	0	0	0	0	1	0	0
Staphylococcus aureus	0	0	2	1	0	1	0
Bacillus cereus	0	0	0	3	0	6	0
Pseudomonas aeruginosa	0	2	1	0	0	0	1
Enterobacter spp	0	0	0	0	1	0	0

Looking at the variety of analyzed food items, 25.45% of bacteria were found in momo's sauce, where 64.28% were *Escherichia coli*. Similarly, *E. coli* was found to be in highest numbers in chowmein as well (60.56%) as in Thakali set (75.35%) and samosa (60.68%). Among the total bacterial isolates, 8.46% were found in pizza in which *Bacillus cereus* was the prominent bacteria (55.63%). Of the total bacteria isolated from burgers, 50.28% were

Bacillus cereus. Pseudomonas aeruginosa was the least detected bacteria in momo's sauce, chowmein, and samosa (14.28%, 8.62% and 19.03% respectively). Our results comply with the findings of Arjyal and Manandhar[13] which has shown the highest occurrence of *B. cereus* in bakery food items in comparison to dairy products and rice items tested in Kathmandu. *B. cereus* was one of the predominant bacteria in ready-to-eat rice items analyzed in Kathmandu.[14] An earlier survey of street foods in Kathmandu has shown that bacterial contamination was detected in all the food samples analyzed. *S. aureus* was found to be the dominant bacteria followed by *Bacillus alvei*, *E. coli* and others.[15] *B. cereus* was the most prevalent bacteria, present in 37.5% of ready-to-eat rice sold in both local and high standard restaurants in Benin city in Nigeria along with three other bacteria *S. aureus*, *E. coli* and *Klebsiella pneumoniae.*[16]

More than one-third of the food samples analyzed in the Brazilian city of Sao Paulo were found to be inappropriate for human consumption due to critical bacterial burdens; *B. cereus*, *S. aureus* and *E. coli* were found in 12.5%, 2.5% and 22.5% of the samples respectively.[17] Staphylococcal foodborne disease outbreaks have proved to affect many people at different times, for example, an incidence of low-fat milk contamination at a dairy items production plant which affected more than 13,000 people in 2000 in Japan.[18] Enterotoxigenic *S. aureus* and *Shigella* spp. were found to be prevalent in raw street vended Indian foods like coriander sauce, of ready-to-eat salads and coconut slices.[19]

A study conducted in Thailand in ready-to-eat food items showed that they were contaminated with *B. cereus*, and *Clostridium perfringes* whereas a heavy load of *S. aureus* was detected in one of the food items Yum pla tuu.[10] In a study conducted in ready-to-eat foods in Iran, *E. coli*, *B. cereus*, *Salmonella* and *S. aureus* were detected and 14% of all samples were contaminated by *Salmonella*.[20] Similar studies conducted in Bangladesh observed that 44.5% of 110 food samples from school-based canteens and local hotels were unacceptable (total coliforms 100/g or ml), whereas the remaining were satisfactory. The laboratory examination indicated that food samples like Jhalmuri (59.1%), sliced fruit (54.2%), vajavuji (53.3%), chotpoti (29.4%) and sarbat (100%) were unsatisfactory, whereas achar (pickles) and ice cream were satisfactory.[21] *S. aureus*, *Salmonella* spp. and *Bacillus cereus* have also been found to be the major bacterial agents found in restaurant food items in Gulf states.[22] Another study performed in raw vegetable salads offered at hotels and restaurants in Bharatpur, Nepal, *Salmonella spp* were found in 35.2% of the 216 samples and *E. coli* were found in 13.4% of the samples.[23] Unhygienic practices like not wearing gloves while preparing and serving foods, directly touching the food items with hands was observed among the vendors in a study conducted in fried rice samples in Kathmandu district.[14]

The total bacterial count in different RTE food items tested ranged from $7x10^5$ to $11x10^2$ cfu/ml (momo: $7x10^5$ to $18x10^2$, chowmein: $7x10^5$ to $11x10^2$, pizza: $5x10^5$ to $12x10^2$, thakali set: $5x10^5$ to $22x10^2$, burger: $6x10^5$ to $13x10^2$, samosa: $5x10^5$ to $19x10^2$). The Department of Food Technology and Quality Control (DFTQC) of the Government of Nepal has set the permissible standard for microbial counts for drinking water and different food items, though not specifically for RTE foods. If we compare the closest food items' category with the DFTQC standard, i.e. of luncheon meat items, all 55% of total RTE food samples tested in our study that showed the presence of bacteria are unsuitable for consumption. The total plate count permissible is 1000 per gram with absence of *E. coli*, *Salmonella* spp, *S. aureus*, *Clostridium perfringens*, *Clostridium botulinum* per 25 gram of food.[8]

The studies on bacterial agents in RTE food items have focused on the detection of levels of different types of toxins causing food borne illnesses[24,25] and the detection of emetic and entero-toxin encoding genes.[26,27] However, some studies have also looked into the antibiotic susceptibility of the isolated bacterial agents.[23,28,29,30,31]

Antibiotic susceptibility pattern of the two most prevalent bacteria in the food items tested in this study have been shown in Table 2 and Figure 4.

Table 2 shows that *E. coli* isolated from different food samples were 100% sensitive to antibiotics polymyxin-B, amoxicillin-clavulanic acid, ceftriaxone, nitrofurantoin and tigecycline and 100% resistant to ampicillin and nalidixic acid. 28 of the 29 *E. coli* isolates (96%) were sensitive to meropenem, cotrimoxazole, azithromycin and chloramphenicol.

All four of the *Pseudomonas aeruginosa* isolates were susceptible to polymyxin-B, ciprofloxacin, cotrimoxazole and azithromycin whereas all four isolates were resistant to ampicillin, nalidixic acid, nitrofurantoin and chloramphenicol.

Table 2: Antibiotic susceptibility pattern of Escherichia coli.

S.N.	Antibiotics used	Sensitive	Intermediate	Resistant
1	Polymyxin-B (300 units)	29(100%)		
2	Ciprofloxacin (5μg)	28(96%)	1(4%)	
3	Meropenem (10μg)	28(96%)	1(4%)	
4	Amoxycillin-clavulanic acid (30μg)	29(100%)		
5	Cotrimoxazole (25μg)	28(96%)	1(4%)	
6	Azithromycin (15μg)	28(96%)	1(4%)	
7	Chloramphenicol (30μg)	28(96%)	1(4%)	
8	Ceftriaxone (30μg)	29(100%)		
9	Nitrofurantoin (300μg)	29(100%)		
10	Tigecycline (15μg)	29(100%)		
11	Ampicillin (10μg)			29(100%)
12	Nalidixic acid (30μg)			29(100%)

The seven isolates of *Salmonella* spp found in this study were all resistant to ampicillin and nalidixic acid whereas all 7 isolates were sensitive to ciprofloxacin, meropenem, azithromycin, chloramphenicol and ceftriazone. In another study conducted in raw vegetable salads in Bharatpur, Nepal, multi-drug resistance was detected in 13.6% *Salmonella spp* and in 13.8% of *E. coli* among which 7.6% of *Salmonella spp* and 13.8% of *E. coli* were Extended Spectrum Beta Lactamase (ESBL) producers.[23]

A single *Klebsiella pneumoniae* isolate was detected in the study which was resistant to ciprofloxacin, amoxycillin-clavulanic acid and nitrofurantoin indicating that this isolate was multi-drug resistant (MDR). Other antibiotics like cotrimoxazole, ceftriaxone, azithromycin, tigecycline, meropenem, chloramphenicol and polymyxin-B were found to be effective for this isolate. Further testing demonstrated that this isolate was ESBL (Extended Spectrum β-Lactamase) producer as well.

One *Enterobacter* spp isolated was resistant to ampicillin; sensitive to ciprofloxacin, azithromycin, tigecycline, meropenem, chloramphenicol, nitrofurantoin and polymyxin-B and intermediate sensitive to cotrimoxazole, ceftriaxone and nalidixic acid.

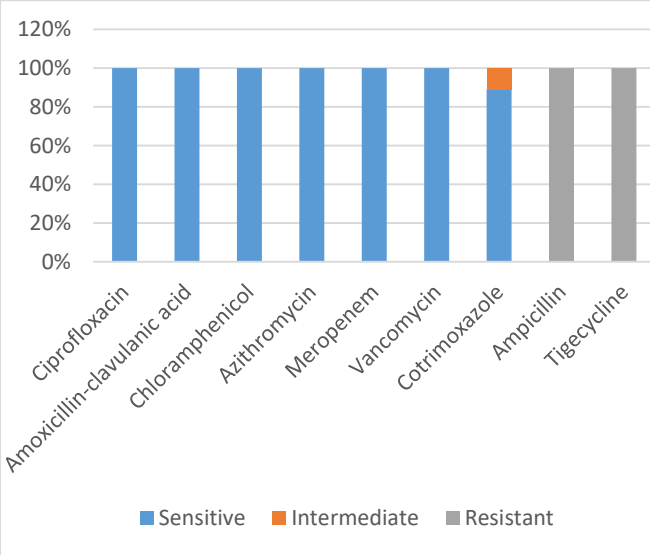

Fig. 4: Antibiotic susceptibility pattern shown by *Bacillus cereus.*

Figure 4 shows that all of the 9 *Bacillus cereus* isolates were resistant to ampicillin and tigecycline; all of the isolates were sensitive to ciprofloxacin, amoxicillin-clavulanic acid, chloramphenicol, azithromycin, vancomycin and meropenem. Eight out of nine *B. cereus* isolates were sensitive and one was intermediately sensitive to cotrimoxazole.

In our study, *Staphylococcus aureus* isolates (n=4) were all resistant to ampicillin but all were susceptible to other antibiotics like ciprofloxacin, cotrimoxazole, azithromycin and chloramphenicol. *S. aureus* isolated from retail meat and its products in China[28] and different retail food products in Denmark[31] have shown higher resistance to penicillin.

Conclusions

The increasing trend of people eating ready-to-eat food items prioritizes the need of such food items be prepared and served in hygienic conditions with no presence of pathogenic bacteria. The presence of *E. coli* and other coliforms (*Klebsiella spp* and *Enterobacter spp*) in tested food samples indicate poor hygienic conditions. The presence of *Staphylococcus aureus* and emetic- and entero-toxin producing *Bacillus cereus* is equally concerning. Poor personal hygiene maintained by food handlers as shown by the presence of normal bacterial flora in foods also reiterates the importance of regular monitoring of food preparation practices and facilities by the concerned authorities, the need to provide regular training to people involved and also to raise public awareness. The antibiotic susceptibility pattern shows that the morbidity caused by community-associated antibiotic resistant bacteria is spreading which needs to be taken into consideration to minimize the development of multi-drug resistance. It is imperative that the food safety regulatory system is in place and actively enforces the defined national standards to ensure that the ready-to-eat food items available in the market is of hygienic quality.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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